TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket Number 01975.0025
		U.S. Application No. 09/582342
International Application. No. PCT/EP98/08522	International Filing Date December 17, 1998	Priority Date Claimed December 24, 1997

Title of Invention: PREPARATION OF CELLS FOR PRODUCTION OF BIOLOGICALS

Applicant(s) For DO/EO/US: Rudi BRANDS

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [X] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [ ] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.

16. [ ] Other items or information:
    a. [ ] Verified Small Entity Statement.
    b. [ ] Copy of Notification of Missing Requirements.

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NUMBER
09/582342	PCT/EP98/08522	01975.0025

430 Rec'd PCT/PTO 23 JUN 2000

17. [X] The following fees are submitted:	CALCULATIONS
Basic National Fee (37 CFR 1.492(a)(1)-(5)):	
Search Report has been prepared by the EPO or JPO.....$840.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$670.00	
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$690.00	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$970.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....$ 96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$840.00
Surcharge of $130.00 for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

Claims	Number Filed	Number Extra	Rate	
Total Claims	21-20=	1	X $18.00	$18.00
Independent Claims	1 - 3=		X $78.00	$
Multiple dependent claim(s) (if applicable)			+$260.00	$
		TOTAL OF ABOVE CALCULATIONS =		$858.00
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28)				$
		SUBTOTAL =		$858.00
Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).			+	$
		TOTAL NATIONAL FEE =		$858.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).		$40.00 per property	+	$
		TOTAL FEES ENCLOSED =		$858.00
		Amount to be refunded		$
		charged		$

a. [X] A check in the amount of **$858.00** to cover the above fees is enclosed.

b. [ ] Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-0916. A duplicate copy of this sheet is enclosed.

The Commissioner is hereby authorized to charge any other fees due under 37 C.F.R. §1.16 or §1.17 during the pendency of this application to our Deposit Account No. 06-0916.

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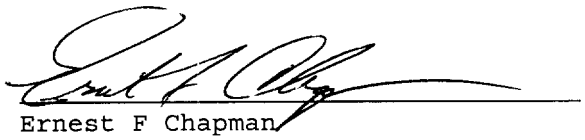

Ernest F Chapman
Reg. No. 25,961

Submitted: June 23, 2000

PATENT
Attorney Docket No. 01975.0025-00000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Rudi BRANDS	
Serial No.: Unassigned	Group Art Unit: Unassigned
U.S. National Stage Application of: PCT/EP98/08522	Examiner: Unassigned
PCT Filed: December 17, 1998	
National Stage Entry: June 23, 2000	
For: PREPARATION OF CELLS FOR PRODUCTION OF BIOLOGICALS	

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination of the above-identified national stage application, please amend this application as follows:

IN THE SPECIFICATION:

In the Abstract, item (57), line 1: delete "up";

In the Abstract, item (57), line 2: replace "till" with -- to --;

Page 1, line 8: replace "an" with -- a --;

Page 2, line 2: delete "up till" and insert therefor -- to --;

Page 2, line 10: delete "up till" and insert therefor -- to --;

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Page 2, line 25: after "substrate" add -- . --;

Page 4, line 1: replace "much less" with -- fewer --;

Page 4, line 27: replace "need" with -- needs --;

Page 4, line 33: after "invention" add -- . --;

Page 5, line 15: replace "As a" with -- A --;

Page 5, line 15: replace "inventions" with -- invention --;

Page 5, line 15: after "can be" delete -- used --;

Page 5, line 15: replace "of" with -- or --;

Page 5, line 20: replace "times" with -- types --;

Page 6, line 9: replace "till" with -- until --; and

Page 9, line 4: after "viable" add -- . --.

IN THE CLAIMS:

Without prejudice or disclaimer, please cancel claims 3-6, amend claims 1 and 2, and add new claims 7-25 as follows:

Claim 1 (Amended). [Method] A method for the preparation of cells for use in the production of [biologicals] a biological, [by] said method comprising culturing cells [up till] to a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:

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a) a first part of the cells of the preproduction batch is used for the preparation of at least one production batch, and

b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

Claim 2, line 1: replace "Method" with -- A method --; and

line 2: insert after "a)" and before "part" -- the first --.

-- 7. The method according to Claim 1, wherein a first preproduction batch is prepared from a working seed stock by at least one passage step.

8. The method according to Claim 2, wherein a first preproduction batch is prepared from a working seed stock by at least one passage step.

9. The method according to Claim 1, wherein the cells are anchorage dependent.

10. The method according to Claim 9, wherein the anchorage dependent cells are derived from hamsters (CHO, BHK-1), monkeys (Vero), bovines (MDNK), canines (MDCK), humans (CaCo, A431), or chickens (CEF).

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11. The method according to Claim 2, wherein the cells are anchorage dependent, are grown on a substrate, and are released from said substrate prior to each transfer step.

12. The method according to Claim 11, wherein the substrate comprises particulate matter or a solid support.

13. The method according to Claim 12, wherein the solid support comprises hollow fibers or micro-carriers or macro-carriers in suspension.

14. The method according to Claim 11, wherein the cells are embedded in a carrier.

15. The method according to Claim 14, wherein the carrier is a Cytodex-3 micro-carrier.

16. The method according to Claim 11, wherein the cells are released from said substrate with a proteolytic enzyme.

17. The method according to Claim 16, wherein the proteolytic enzyme is trypsin.

18. The method according to Claim 16, wherein the cells are treated with PBS and/or EDTA prior to exposure to the proteolytic enzyme.

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19. The method according to Claim 1, wherein the biological is a virus.

20. The method according to Claim 1, wherein the biological is a protein.

21. The method according to Claim 20, wherein the protein is an enzyme.

22. The method according to Claim 1, wherein:

a) the proportion of the cells of the preproduction batch used for the preparation of said at least one production batch ranges from 80% to 90%, and

b) the remaining proportion of the cells of the preproduction batch used as a seed for the preparation of said at least one subsequent preproduction batch ranges from 10% to 20%.

23. The method according to Claim 1, wherein the cells are parked at a certain passage number by exposure to an ambient temperature ranging from 17 to 32 degrees C.

24. The method according to Claim 23, wherein said parked cells are revitalised to log growth by raising the temperature and changing the culture media.

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25. The method according to Claim 1, wherein the cells are frozen at a temperature of less than -80 degrees C. in bulk, and thawed prior to use. --

REMARKS

Claims 3-6 have been cancelled without prejudice or disclaimer, claims 1 and 2 have been amended, and new claims 7-25 have been added to more particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Support for these amendments and new claims can be found, among other places, in the specification and original claims as follows:

Pending Claim	Support
1	Orig. Claim 1
2	Orig. Claim 2
7	Orig. Claim 3
8	Orig. Claim 3
9	Orig. Claim 4; Page 2, Line 21
10	Page 5, Lines 12-14
11	Orig. Claim 5
12	Page 3, line 9; Page 5, Line 20
13	Page 5, Lines 20-23
14	Page 5, Line 22
15	Page 6, Lines 5-6
16	Page 5, Line 28
17	Page 3, Line 33
18	Page 5, Line 29

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Pending Claim	Support
19	Orig. Claim 6
20	Page 2, Line 35
21	Page 2, Line 36
22	Page 4, Lines 8-13
23	Page 5, Lines 5-6
24	Page 5, Lines 6-7
25	Page 5, Lines 8-9

Amendments to the specification were made to correct obvious typographical, spelling, and grammatical errors. Care has been taken so that no new matter has been introduced into the application.

Applicant respectfully requests examination and allowance of the amended claims. Applicant submits that the pending claims are in condition for allowance, and requests a prompt, favorable action on the merits.

If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

By: Michele C. Bosch

Dated: June 23, 2000

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Preparation of cells for production of biologicals

The present invention is concerned with a method for the preparation of cells for use in the production of biologicals.

For the production of biologicals on e.g. cell lines, the preparation of large amounts of cells using an scaling up procedure in bioreactors will be necessary.

The US patent No. 5,017,490 discloses such a scaling up procedure which provides in particular the advantage of a low risk of transfer contamination. This method is, however, not suited for anchorage dependent cells (hence, not for cells which only grow if fixed to a substrate) or cells embedded in a substrate (e.g. in porous carriers).

The US patent No. 4,644,912 discloses a method for the preparation of anchorage-dependent cells for the production of biologicals (i.e. viruses) starting with a cell working seed, and with subsequent passages effected in increasing consecutive volumes of 1 litre, 5 litre, 25 litre, 150 litre bioreactors, and finally either in a 1000 litre bioreactor or in a multiplicity of 150 litre bioreactors. In between any of these passage steps the cells were released from their carriers with a dilute protease solution. In the final passage the inoculation by the virus was effected.

Assuming average cell cycle times of about 20-24 hours the passage intervals may be about every 3-5 day. Therefore, in order to expand the cells to sufficient large cultures from a MWCS[1] the total scaling up procedure may take several weeks, depending on the final bioreactor volume.

In the above methods for preparation of cells each of the ultimate production batches has to be prepared from the MWCS. For the production of vast amounts of biologicals it will be necessary to utilise several parallel culturing lines up to the largest vessel volumes. Such preparation procedure, hence, is very time consuming and necessitates the operation of a very considerable number of bioreactors for the preparation of the cells as well as for the production of the biologicals.

It is an object of the present invention to provide a much faster through-put in preparation of cells for the production of biologicals.

[1] MWCS = manufacturer's working cell bank

Accordingly, the present invention relates to a method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:

a) part of the cells of the preproduction batch is used for the preparation of at least one production batch, and

b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

More in particular, the present invention relates to a method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:

a) part of the cells of the preproduction batch is transferred to be used for the preparation of at least one production batch, and

b) the remaining part of the cells of the preproduction batch is transferred to be used as a seed for the preparation of at least one subsequent preproduction batch.

In a preferred embodiment of the present invention the first preproduction batch is prepared from a working seed stock by at least one passage step.

In a further preferred embodiment of the present invention the cells which are prepared are anchorage-dependent. In the latter case it will generally be necessary that the cells are grown on a substrate. It will then be advisable during the repeated process each time when part of a batch is used for the preparation of a new batch to add an additional amount of substrate. In a preferred embodiment, each time prior to the addition of substrate at least part of the cells are first released from their original substrate

As used herein the expression "production batch" means a culture of cells which is employed for the production of biologicals.

As used herein the expression "preproduction batch" means a culture of cells which is used in the process according to the present invention for the preparation of at least one production batch (as defined above) and one subsequent preproduction batch.

As used herein the expression "biological" means any substance or organism which can be produced from a cell culture. Examples of "biologicals" are viruses and proteins such as enzymes.

As used herein the expression "working seed stock" means an amount of a certain type of cells of defined ancestry stored to be used as a seed from which all cultures of the same type of cells are derived.

As used herein the expression "anchorage-dependent cells" means cells which for their proper growing and/or propagation need to be attached to a substrate as defined herein.

As used herein the expression "substrate" means any particulate matter useful for the attachment of cells.

As used herein the expression "passage step" means a sequence of activities in the propagation and production of cells comprising at least the transfer of a suitable amount of cells and of a suitable amount of culturing medium into a production vessel, the incubation of the vessel at conditions suitable for the growing and propagation of the cells during a time sufficient for effective growing and propagation of the cells. Optionally a passage step may comprise separation of the cells from the culture medium and/or from the substrate after a time sufficient for effective growing and propagation of the cells.

It will be clear to the man skilled in the art that the method according to the present invention differs essentially from methods known in the art wherein cells are produced in a continuous process rather than the present discontinuous process. According to the patent publications EP0417531 and WO89/08701 continuous culture systems can be employed for the production of viruses as well. Firstly cells are grown in a first bioreactor, and after a certain cell density is reached cells are fed continuously from said first bioreactor into a second bioreactor. In this second bioreactor viruses are grown on the cells and subsequently these viruses are withdrawn continuously from this second bioreactor.

The basic method of working according to the present invention is to use a mother bioreactor from which the production bioreactor(s) is (are) fed with cells. When the cells are anchorage dependent, after each passage step cells preferably need to be detached from their substrates.
A trypsinisation procedure on large bioreactors has been developed for this purpose.
The production cells are defined up to a specific and characterised passage number for a so-called ECB[2]. The method described allows high through-put production since the up scaling

[2] ECB = Extended Cell Bank

route from WCS to production cells can be very much shortened and much less bioreactors are needed since parallel production lines are not needed anymore.

Various embodiments of the present invention are depicted in Figure 1.

In a preferred embodiment cells are expanded from one ampoule of a MWCS up to the level of the first preproduction batch through one or more passage steps. The size of the bioreactor used for such a preproduction batch can range from several litres working volume to several hundreds of litres. Next, a part e.g. 10-20 % of the cells thus expanded (e.g. passage X) are used to repopulate a bioreactor for the production of a subsequent preproduction batch (being passage number X+1), whereas the bulk of the cells is transferred (passage X or X+1) to a larger bioreactor size in order to start production directly or to first populate it, and subsequently start production.

In classical serial production lines the number of doubling of the cells derived from the MWCS at the moment of harvest is known up front within certain limits. A maximum allowable generation number is set to the production system at the onset.

In the method according to the present invention the maximum number of cell passages can be defined by ECB. Production passage number (the number of cell passages used prior to production of the biological product), hence, is irrelevant within the limits set by ECB. As a consequence, such maximum number of passages is to be obeyed in view of regulatory restrictions. As a result the particular batch of produces biologicals is the end product of one direct scaling up route.

In order to verify whether the specifications of the cells at the stage of ECB in production are similar to the MCB[3] one need to perform specific validation for this purpose with respect to growth characteristics, freedom of adventitious, extraneous and endogenous agents at the different stages, karyology iso-enzyme analysis and so on. Once such ECB is fully characterised one may allow to produce the product with cells at any passage number between MCB and ECB, since it may be assumed that cells have not changed in between in their specs. As a result tests on the MWCS therefore can be limited to sterility testing. This is a particular advantage of the method according to the present invention

[3] MCB = Master Cell Bank

With the maximum passage number set one may use cells at any stage in between. From this in order to further minimise the time needed to expand the cells from the MWCS to production bioreactor it would be an advantage to enable bulk start-up of cells. This can be done for example in one of the following ways:

- Cells may be parked at a certain passage number during longer intervals at ambient temperature (17-32 °C) and be revitalised to log expansion growth by raising the temperature and changing the culture medium, or
- Cells may be frozen (Temp < -80°C) in bulk and be thawed prior to transfer them to a pre-set volume bioreactor, thereby reducing the needed up scaling route significantly.

The method according to the present invention can be carried out with animal cell cultures and more in particular with anchorage dependent cells. Suitable types of cells are e.g. hamster cells (CHO, BHK-1), monkey cells (Vero), bovine cells (MDBK), canine cells (MDCK), human cells (CaCo, A431) or chicken cells (CEF).

As a bioreactor according to the present inventions can be used a single unit of a plurality of units of e.g. stirred fermenters, fixed bed fermenters, fluidized bed fermenters, air lift fermenters, or a hollow fibre reactors.

Cells of the above times can and some even should be cultured when fixed to a solid support, like micro-carriers or macro-carriers in suspension, e.g. in a fixed bed, a fluidized bed or in suspension, or like hollow fibres. Cells can also be embedded into a carrier (e.g. porous carrier)

In the course of the method according to the present invention, in particular when using a solid support, cells are to be released from this solid support. This can be effected by any method useful for detaching of cells from a solid support. Advantageously, to this end use can be made of a proteolytic enzyme solution. Optionally, this enzymatic release step can be preceded by one or more pre-conditioning steps, e.g. by treatment with PBS and/or EDTA, in order to enhance the proteolytic efficiency, and/or in order to reduce the amount of proteolytic enzyme required.

EXAMPLE 1

Cell detachment and separation from carriers prior to transfer to next bioreactor

Anchorage dependent cells of a MDCK[4] cell line were cultured at 37 °C on Cytodex-3 micro carriers (Pharmacia, Uppsala, Sweden) (5 g of carriers/l) in a stirred bioreactor of 4 litre ("mother bioreactor"). The growth medium was EpiSerf (Life Technologies, Paisly, Scotland). Growth was continued till a maximum of $5x10^6$ cells/ml of culture.

The cells were detached from the carriers by trypsinisation in a Trypsin-EDTA solution (Life Technologies, Paisly, Scotland).

After settling of the carriers 80% of the detached cells were transferred to 3 other bioreactors of similar size. The latter "production" bioreactors all have carriers (cell substrate) added to them up front. Cells were allowed to repopulate the carriers and subsequently used for production in these production bioreactors.

The remainder of the cells in the "mother bioreactor" were allowed to repopulate the remaining Cytodex-3 carriers and were cultured to the desired cell density.

EXAMPLE 2

Cell detachment without separation from carriers prior to transfer to next bioreactor

The culturing of cells was carried out as described in Example 1, however after trypsinisation 80% of the detached cells including the carriers are transferred to the 3 production bioreactors. Additionally, suitable carriers were added to all bioreactors.

[4] MDCK = Madin Darby Canine Kidney (cell line)

EXAMPLE 3

Cell detachment without separation from carriers after transfer to next bioreactor

The culturing of cells was carried out as described in Example 1, however, 80 % of still adhered cells were transferred to a bioreactor of similar size which next was used directly for product generation.
The remaining cells on micro carriers in the mother fermenter were next detached by trypsinisation, where after new carriers were added and cells were allowed to repopulate the substrates.

EXAMPLE 4

Start-up from frozen bulk cells

In this experiment part of the culture was used to rebatch the mother fermenter and some daughter fermenters and part of the culture was used to freeze cells in bulk.
Frozen bulk cells (total $14.4X10^8$ cells) were inoculated in a start culture in a 3 litre mother fermenter containing 5 g Cytodex per litre and EpiSerf medium, and thereafter incubated at 37 °C. Residual cryo-preservatives were removed by a medium change on day 1.
At day 2 trypsinisation was carried out, 50% of the cells were bulk frozen and the remaining cells were inoculated to micro-carriers in a subsequent fermenter.
From Table 1 it can be deduced that the cells do continue to grow at a normal rate between day 2 and 3
On day 4 the content of the mother fermenter was trypsin-detached and rebatched onto new micro-carriers (10 g/l) in two other fermenters next to the mother fermenter.
At day 5 the plating efficiency turned out to be about 85%.

Table 1

day	3 litre mother fermenter cells x 100.000/ml	3 litre fermenter cells x 100.000/ml	3 litre fermenter cells x 100.000/ml
0	NOD		
1	6.6		
2	14		
3	15.5		
4	30		
5	5.5	10	10
plating efficiency	85%	85%	85%

EXAMPLE 5

Transfer from small scale mother fermenter to large scale production fermenter

Cells were scaled up to a large scale in 65 litre and 550 litre fermenters (50 litre and 250 litre working volume, respectively) using a micro-carrier density of 5 g Cytodex per litre.

As can be seen from Table 2, 90% of the total of cells is transferred to the large scale fermenter from a 50 litre fermenter culture with 800.000 cells/ml of which 69% proved to be viable.

The same was found in the 50 litre mother fermenter; about 69% of the repropagating cells turned out to be viable.

The procedure was as follows:

On day 0, the carriers were allowed to settle in the 50 litre culture, where after the supernatant (culture medium) was removed and replaced by PBS. The content of the fermenter was agitated for 5-15 minutes. The supernatant was removed after resettling of the carriers. This step can be repeated if needed.

Next this step was repeated with PBS/EDTA (0.4 gram EDTA/litre PBS). Again the culture was agitated during 5-15 minutes, carriers were allowed to settle, the supernatant was removed, and the PBS/EDTA step was repeated until cells had become rounded and were ready to be trypsin-detached.

Then trypsin (0.025% final concentration) was added to the PBS/EDTA and incubated for 5-15 minutes. Next either the cell containing supernatant (after settling of now "nude" carriers) were transferred (as in example 9) or the mixture of cells plus carriers were transferred (total 80 % of total mix).

After transfer of the cells to the 550 litre fermenter the remainder of the cells (hence, 10% of the viable cells) were allowed to repopulate the carriers still present in the fermenter after refilling the 50 l fermenter with culture medium.
About 70% of the cells proved to be viable

Table 2

day	50 litre culture cells x 100.000/ml	250 litre culture cells x 100.000/ml
0	8 (400 x 10^8 total cells)	1.1 (275 x 10^8 viable cells)
1		0.8
2		2.9
3		3.4
4		8.9
5		18.0

EXAMPLE 6

Analogous to Example 5, however, 80% of the culture of the carrier-bound cells were transferred from the mother bioreactor to the production bioreactor. Production was started after addition of virus.
The 20% of cells and carriers remaining in the mother bioreactor were trypsinized and detached and upon addition of new substrate into the mother bioreactor were allowed to repopulate the mother bioreactor while production is ongoing in the physically separated production bioreactor.

EXAMPLE 7

Large scale culture started from bulk frozen cells.

Bulk frozen cells were thawed and inoculated on a 10 litre (working volume) fermenter (Cytodex carrier density 5 g/l; culture medium EpiSerf) at an inoculation density of $1x10^6$ cells/ml. After attachment, the culture medium was replaced in order to remove residual cryo-protectants.
After day 1 the amount of viable cells attached to the carriers was $0.45x10^6$ cells/ml which from then on started growth. At a density of $2.8x10^6$ cells/ml the cells were detached from their

carriers by trypsinisation and 80 % was transferred to a 50 litre working volume fermenter (carriers 5 g/l).

As can be deduced from Table 3, at day 1 the amount of viable cells after bulk freezing of cells was about 45 %.

Of the total amount of transferred cells, the viability after trypsin detachment was 71.4%.

Table 3

day	cell density (x 10^6/l) in:	
	10 litre fermenter	50 litre fermenter
0	**1.0**	
1/2	**0.45**	
3/4	**1.3**	
5	**2.6**	
6	**2.8** (280 x 10^8 total)	
6	**0.6** (60 x 10^8 total)	**0.28** (140 x 10^8 total)
7		**0.4** (200 x 10^8 total)

Claims

1. Method for the preparation of cells for use in the production of biologicals, by culturing cells up till a desired cell volume of a preproduction batch, where after in a repeated discontinuous process:
   a) part of the cells of the preproduction batch is used for the preparation of at least one production batch, and
   b) the remaining part of the cells of the preproduction batch is used as a seed for the preparation of at least one subsequent preproduction batch.

2. Method according to claim 1 wherein in the repeated discontinuous process:
   a) part of the cells of the preproduction batch is transferred to be used for the preparation of at least one production batch, and
   b) the remaining part of the cells of the preproduction batch is transferred to be used as a seed for the preparation of at least one subsequent preproduction batch.

3. Method according to claim 1 or 2, characterised in that a first preproduction batch is prepared from a working seed stock by at least one passage step.

4. Method according to claim 1-3, characterised in that the cells are anchorage-dependent.

5. Method according to claim 2, characterised in that the cells are anchorage dependent, the cells are grown on a substrate, and prior to each transfer step the cells are released from their substrate.

6. Method according to claim 1-5, characterised in that the biological of interest is a virus.

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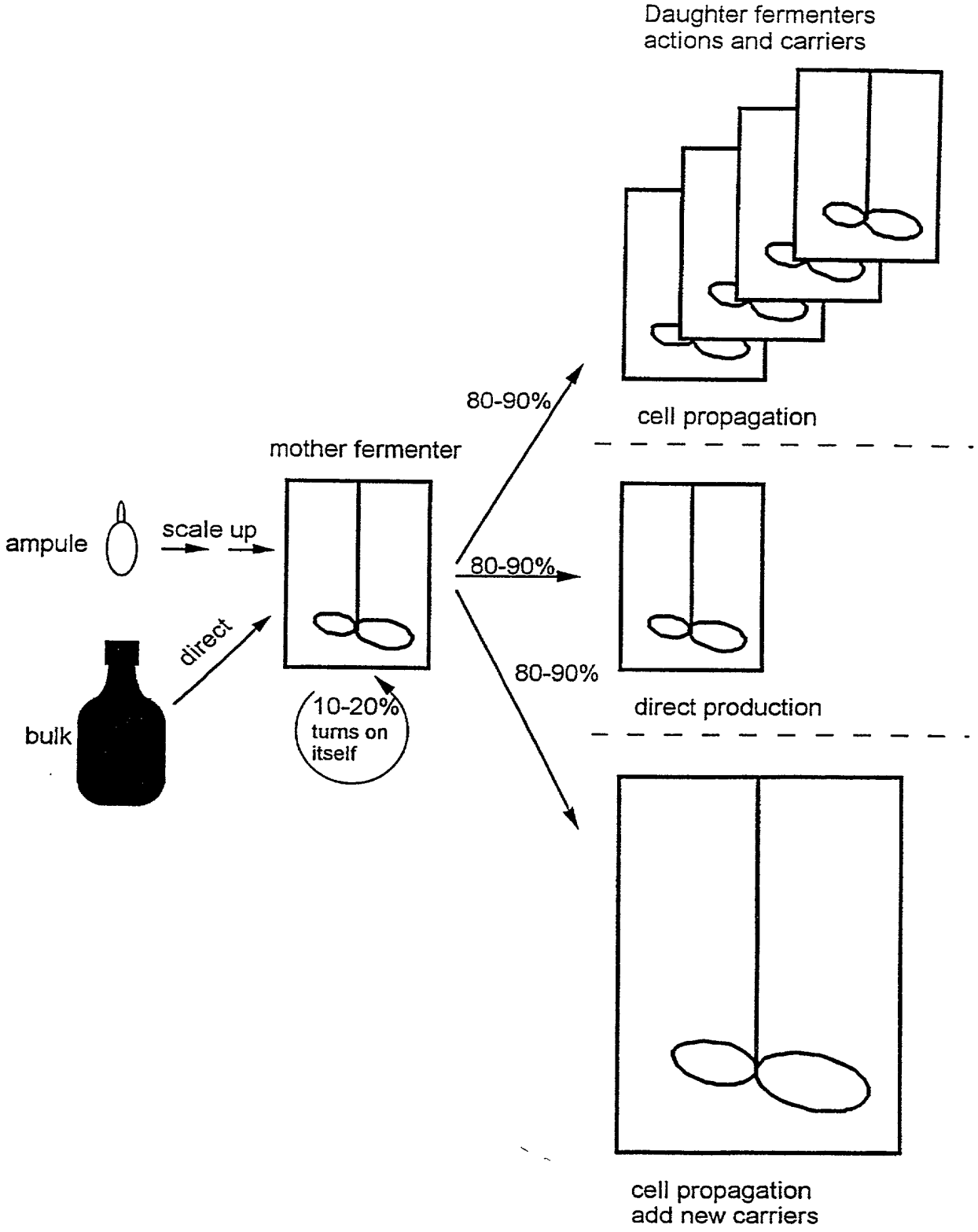
FIGURE 1



Attorney Docket No.: DIR 0550 US
Declaration/Power of Attorney
Worldwide Rights

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I/We hereby declare that: my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first, and sole inventor (if only one name is listed below) or an original, first, and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: Preparation of cells for production of biologicals
the specification of which ☜ is attached and/or ☜ was filed on _____ as United States Application Serial No. _____ or PCT International Application No. PCT/EP98/08522 and was amended on _____.

I/We hereby state that I/We have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I/We acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I/We hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate or § 365(a) of any PCT international application(s) designating at least one country other than the United States, listed below and have also identified below, any foreign application(s) for patent or inventor's certificate, or any PCT International application(s) having a filing date before that of the application(s) of which priority is claimed:

Country	Application Number	Date of Filing	Priority Claimed Under 35 U.S.C. 119
EP (NL designated)	97204110.7	24 December 1997	× YES NO

I/We hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Number	Date of Filing

I/We hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s) or § 365(c) of any PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application(s) in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application(s) and the national or PCT International filing date of this application:

Application Number	Date of Filing	Status (Patented, Pending, Abandoned)

I/We hereby appoint the following attorney and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith. **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.,** Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; Roger D. Taylor, Reg. No. 28,992; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Dirk D. Thomas, Reg. No. 32,600; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W. Edmondson, Reg. No. 33,871; Michael R. McGurk, Reg. No. 32,045; Joann M. Neth, Reg. No. 36,363; Gerson S. Panitch, Reg. No. 33,751; Cheri M. Taylor, Reg. No. 33,216; Charles E. Van Horn, Reg. No. 40,266; Linda A. Wadler, Reg. No. 33,218; Jeffrey A. Berkowitz, Reg. No. 36,743; Michael R. Kelly, Reg. No. 33,921; and James B. Monroe, Reg. No. 33,971; and _____. Please address all correspondence to **FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.** 1300 I Street, N.W., Washington, D.C. 20005, Telephone No. (202) 408-4000.

I/We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

IN TESTIMONY WHEREOF, I/We have hereunto set our hands.

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